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Synthesis and Purification of a Nucleoside Platinum Complex That Promotes DNA Cross-Linking

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Abstract

Cancer is a deadly and ever present disease in humans and its treatment has become a focus of many types of research. This project was designed to synthesize a platinum complex that will cross-link DNA and in turn cause cell death for rapidly dividing cells (usually cancer cells). The target compound strongly resembled the structure of a nucleic acid, thymidine. Further, nucleic acids are extremely important to cell life and cell membranes have transporters specific for them. Therefore, the purpose to exploring this new platinum complex was to create compound that a cell can easily transport inside its cell and nuclear membranes. With an end product structure in mind, a series of seven reactions were attempted in the lab in order to create this compound. Upon purification by column chromatography, each intermediate was tested for structure and purity using nuclear magnetic resonance spectroscopy (NMR) and thin-layer chromatography (TLC).

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Introduction

Cancer is defined as the disease caused by an uncontrolled division of abnormal cells in an organ or section of the body [7]. Unfortunately, cancer is everywhere in our world and kills millions of humans a year. Although, within the last fifty years our knowledge of cancer and our ability to combat this deadly disease has increased significantly. Current drugs or treatments, such as cisplatin and other forms of chemotherapy, have allowed certain types and early stage cancer to be considered curable diseases. One of the key mechanisms utilized by many of these anti-cancer drugs or treatments is the disruption of the cell's DNA integrity. DNA, or deoxyribonucleic acid, is the genetic material needed for proper cell function, growth, and replication [1]. Accordingly, cells dedicate many resources and mechanisms in order to maintain the reliability of their genetic code through costly repair and signaling pathways [2].In addition, if the DNA of a cell is altered or destroyed beyond repair, most cells will experience G2 arrest of the cell cycle and will follow one of two outcomes [2]. First, the cell could continuously attempt methods of repair and deplete its supply of ATP/NADH; thus inadequate resources lead to cell death [8]. This cell killing process is called necrosis. In the second outcome, DNA damage causes signal transduction; thus causing the cell to implement a programed cell death process known as apoptosis. The goal of many anti-cancer drugs is to permanently alter the DNA of a cancer cell and cause cell death via necrosis or apoptosis.

The cisplatin drug mentioned above is a key pharmaceutical anti-cancer drug that modifies DNA structure by cross-linking DNA. As used here, cross-linking is defined as a link between two nucleotide bases. For cisplatin, the cross-link is accomplished by the use of a platinum complex. In order to understand the mechanism behind the anti-cancer drug family of platinum complexes, cisplatin's mechanism of action will be examined in detail. As seen by the structure in Figure 1, cisplatin is a small inorganic molecule with the chemical formula of $Pt(Cl)_2(NH_3)_2$.

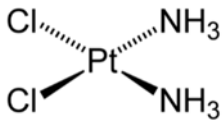


Figure 1: Structure of cisplatin

The cornerstone to the medicinal usefulness of cisplatin and other platinum complexes is the presence of the electrophilic platinum II ion. Specifically, it is this positive platinum II ion that is attracted to the electron rich atoms of DNA molecules, making cross-linking possible [3]. In the case of cisplatin, the drug has affinity for, or prefers, the cross-linking of guanine bases [8]. Specifically, cisplatin binds to the nucleophilic N7 of two guanine residues (Fig. 2a). It should be noted that the geometry of the platinum complex is most effective in the cis, not trans, stereoisomer (Fig. 2b).This is because the trans stereoisomer causes inter-strand binding which is more easily repaired than the inter-strand binding caused by the cis stereoisomer [1].

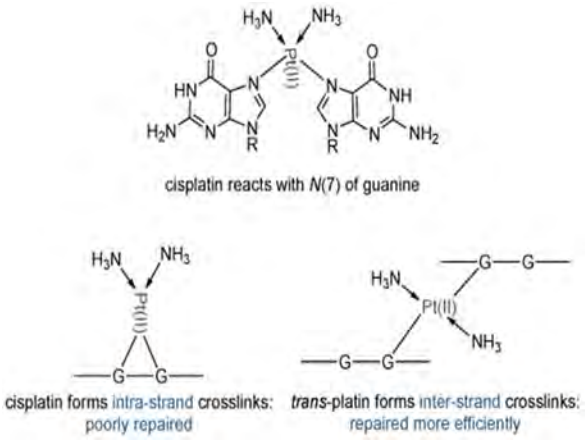


Figure 2: 2a) Cisplatin forming a cross-link with the N7 nitrogens of two guanine residues. 2b) The intra-strand versus inter-strand DNA cross-links of cisplatin

Once the platinum is covalently bonded to the nitrogen atoms, the steric strain of the base residues being brought into closer proximity creates a “kink” in the DNA. This change in DNA structure disrupts the DNA base stacking; therefore decreasing the stability and even causing unwinding of the DNA helix [5]. The three dimensional “kink” created by cisplatin can be seen in Fig. 3 below [1].

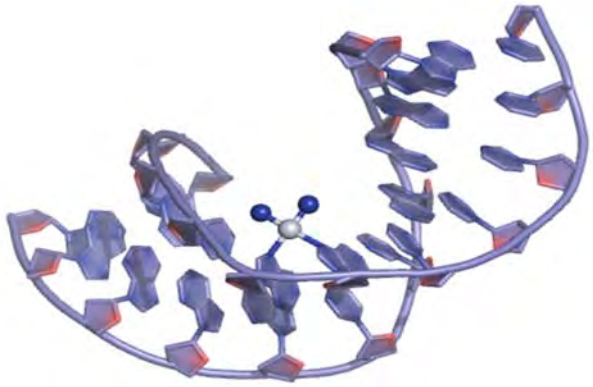


Figure 3: 3D representation of cisplatin cross-linking DNA and disrupting DNA base stacking via creating a "kink"

With the mechanism of cisplatin understood, other platinum II complexes can be introduced. Many other anti-cancer drugs, as seen in Fig. 4, are platinum complexes that use the same DNA cross-linking mechanism as cisplatin [4].

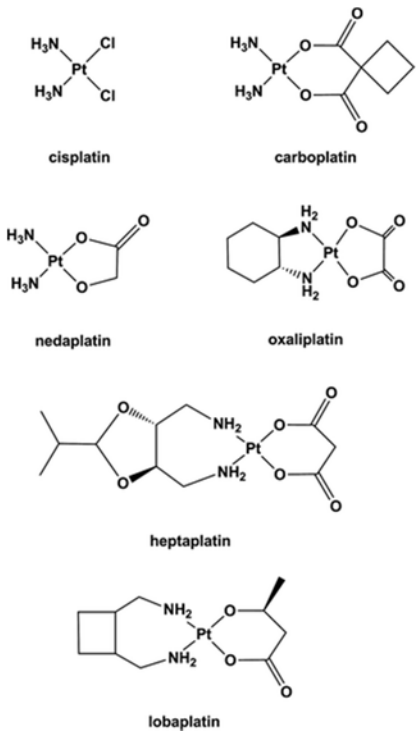


Figure 4: Examples of platinum II complex drugs

In the presence of these types of cross-links, critical DNA replication enzymes are slowed or unable to commence, sustain, and terminate DNA replication. In treatment with low doses of cisplatin, human cells have been found to use the general DNA

damage recovery pathways of mismatch repair and an increase in replicative bypass [2]. With sufficient dosage, human cells are unable to repair the DNA damage caused by cross-linking and succumb to necrosis or apoptosis.

By definition, cancer cells are nearly always replicating; thus are more susceptible to the DNA cross-linking abilities of cisplatin and other platinum complexes. In fact, when cisplatin was first introduced in clinical use, the cure rate of many solid malignancies was increased significantly. Most notable was the cure rate of testicular cancer which was increased from 10% to 85% [8]. It should be noted that despite the ability of cisplatin and other platinum complexes to combat some types of cancer, their effectiveness in other types of cancers is more limited because of acquired or intrinsic resistance [6].

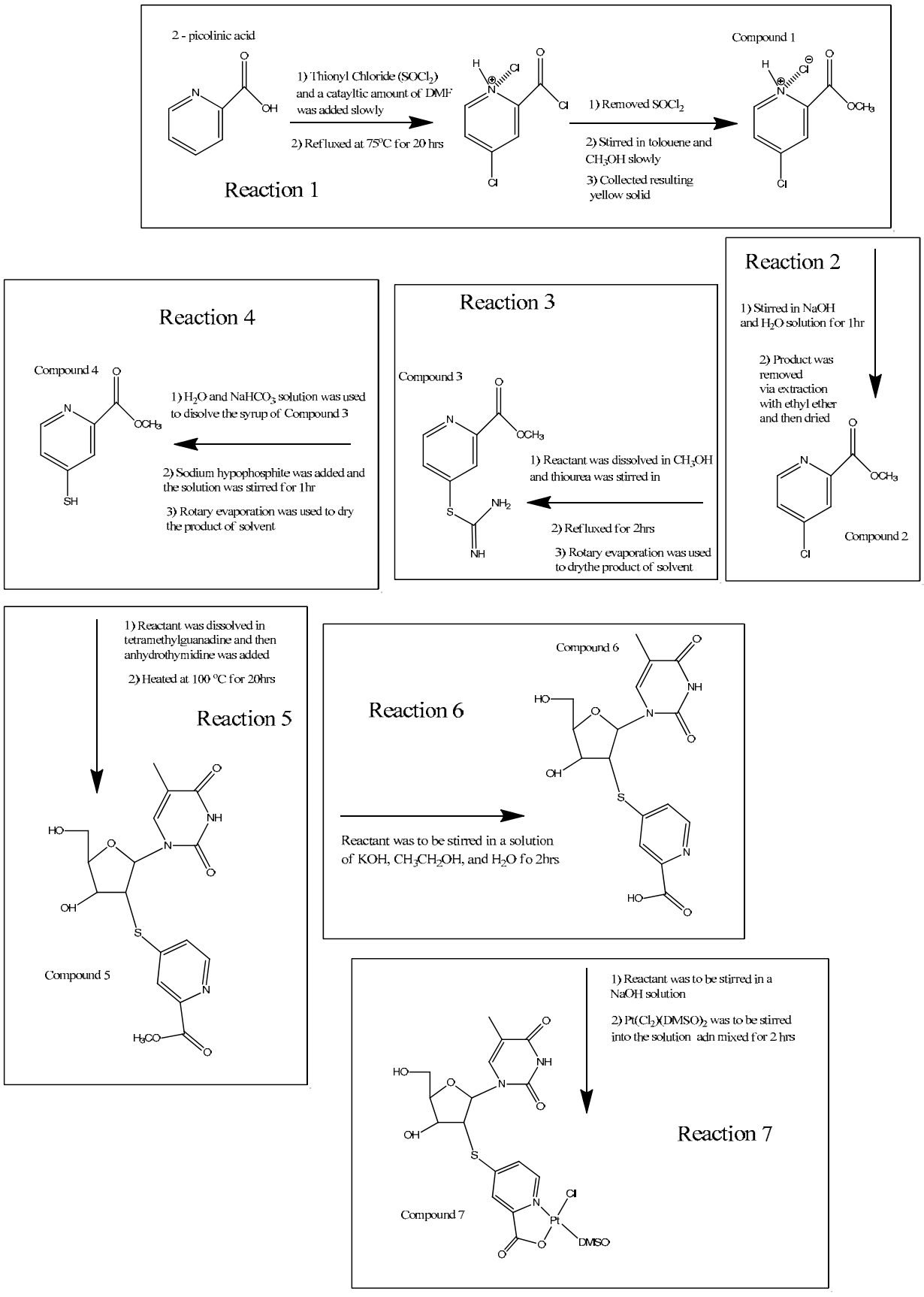
Further rendering the effectiveness of this drug class is the fact that the entirety of molecular mechanisms that underlie human cell's resistance to platinum complexes are poorly understood [4].Specifically, there is a transport problem. Platinum complexes are positively charged inorganic compounds; making successful transportation to the DNA of the cell difficult. The platinum II ion is prone to form bonds and interactions with the nucleophilic atoms of many molecules and proteins of the human body. Therefore, in order to induce DNA cross-linking with these complexes, they are administered to patients intravenously in high dosages. The necessity for high dosages of platinum complexes are known to cause side effects that include: kidney damage, nerve damage, nausea and vomiting, and hear lose [8]. Consequently the ongoing second generation of platinum complex drugs aims to be more effective at lower dosages.

Although several routes could be taken to try to improve upon these drugs need for high concentration, solving the transportation issue is a sought after result. If the drug were some molecule that could easily be up taken by the cell, less concentration would be needed in the form of a direct injection to a tumor.

In line with this realization, the goal of this research project was to synthesize and purify a platinum complex that is incorporated on a nucleoside. It is key to note the

incorporation of the nucleoside. Cells have a multitude of protein transporters designated for nucleosides and nucleotides, as they are the molecular building blocks of DNA, a necessity to cell life. With the inclusion of a nucleoside, the target compound was designed as a pro-drug that would allow transportation of a reactive platinum complex into the cell. As the end goal product of a series of seven reactions, this target compound was a thymidine nucleoside with a platinum II complex coordinated to a picolinic acid residue that is attached to the nucleoside. This attachment is designed to bind platinum-chloride. The intended reactions, intermediates, and end product can be seen in Fig. 5 on page 6. This project's goal was to synthesize this end project and prove its structure from proton and platinum NMR.

Scheme 1: Proposed Reaction Series of Target compound

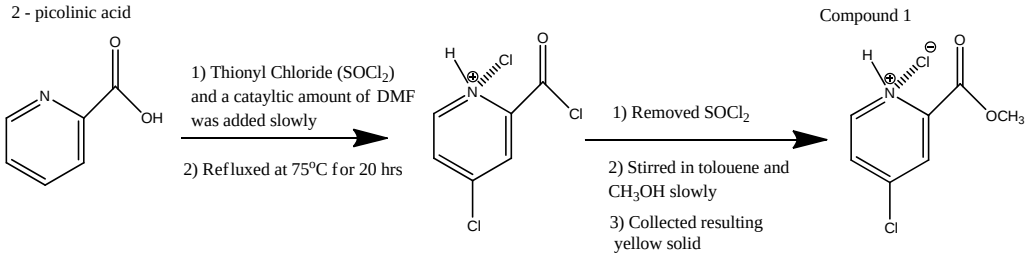


Experimental Methods and Data

Reaction 1 – Synthesis of Compound 1

Reaction 1 was performed in two separate steps. The starting material was picolinic acid (7.015g, 5.686E-2 mol) and was dissolved into thionyl chloride ($SOCl_2$) (24.0mL, 3.304E-1 mol) in a 100mL round bottom flask. The flask was placed in a heating mantle and fit with a water condenser and breathing apparatus. Once dissolved, a catalytic amount (3-4 drops) of dimethylformamide (DMF) was added to the flask. The solution was stirred and heated by the mantle. It was brought to reflux (roughly 75^{o}C) which was maintained for 20 hours. The result of this step was the creation of an acid chloride from the carboxylic acid attachment, creation of an ionic salt via the protonated nitrogen and chlorine ion, and electrophilic aromatic addition of chlorine. Scheme 1 below outlines Step 1 of Reaction 1.

The following day, a standard distillation apparatus was setup in order to remove the excess thionyl chloride from the resulting solution. Immediately after Step1 was stopped at the end of its allowed time, the flask containing the product of Step 1 was used as the distillation flask and distillation began. As distillation proceeded, the collecting flask was slowly filled with thionyl chloride that was being removed from our distillation flask. After the distillation system stopped dripping, signaling the absence of thionyl chloride from our product, the product was kept heated to prevent precipitate formation. While kept warm by a jacketed addition funnel, the product solution was slowly dripped over the course of 20 minutes into a 100mL round bottom flask that contained a stirring solution of 6mL of methanol (CH_3OH) and 6mL of toluene. Once completely added, the stirring solution was placed into an ice bath for 1 hour in order to allow for precipitation of Compound 2. Vacuum filtration was then used to separate and dry the product. In addition to vacuum filtration, the solid product was placed in a clean, dry, and massed 25mL round bottom flask that was placed on a vacuum pump overnight in order to further dry the solid. Step 2 of this reaction created a methyl ester out of the acid chloride group, an esterification reaction. Scheme 1, as seen below, outlines both steps of Reaction 1.



Scheme 2: Reaction 1 – Step 1: Synthesis protonation of aromatic nitrogen to create a salt and addition of chlorine via electrophilic aromatic addition. Step 2: Esterification of the acid chloride group.

In the highest yield of Reaction 1, 3.7199g of Compound 1 (208.044 g/mol) was isolated. This is a percent yield of 31.45%. A proton and carbon NMR were taken and were found consistent with assigned structure. ^{1}H NMR (DMSO, 300MHz): 10.10-9.25 (exchangeable H, 11H, NH); 8.74 (d,5H,CH); 8.22 (s,5H,CH); 7.85 (d,2H, CH); 3.90 (s,8H,CH_3); 2.48 (s, H, DMSO). Figure 6 shows the ^{1}H NMR of Compound 1. ^{13}C NMR (DMSO, 300MHz):164.17 (CH,7C); 151.21 (CH, 2C); 149.03 (C, 6C); 144.19 (C, 4C); 127.41 (CH, 3C); 124.92 (CH, 5C); 52.819 (CH_3, 8C).

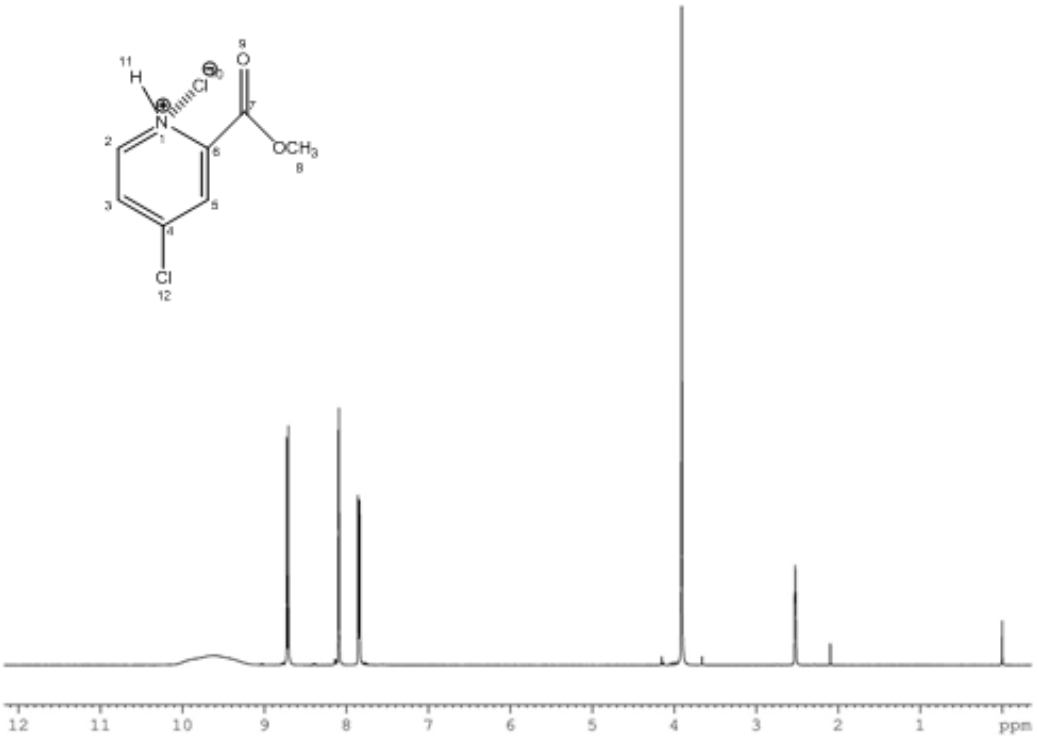
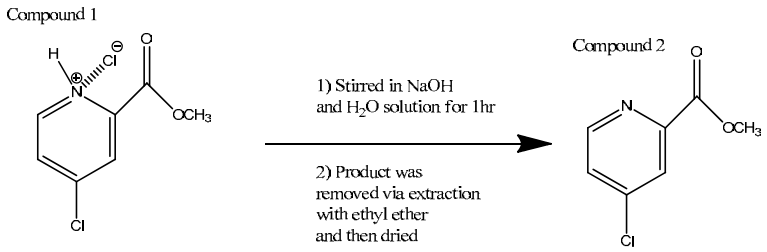


Figure 5: Proton NMR of Compound 1

Reaction 2 – Synthesis of Compound 2

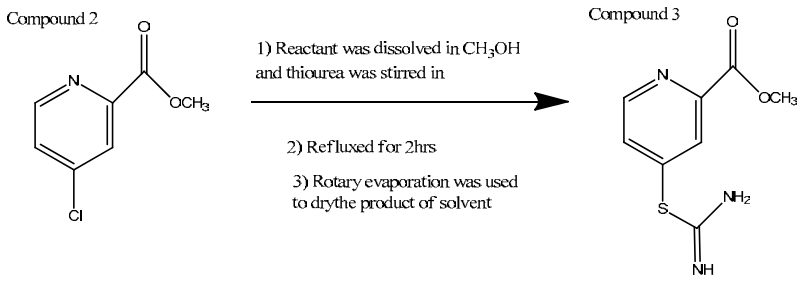
Reaction 2 was taken in order to neutralize the salt, which was done with a strong basic solution of NaOH. Compound 1 (3.6011g, 1.731E-2 mol) was dissolved and stirred in a solution of 25mL of water and NaOH (.7222g, 1.805E-2 mol). A color change occurred (light clear yellow to opaque dark yellow) as the solution was stirred in a 100mL round bottom flask at room temperature for 1 hour. Next, the product of the solution was extracted with diethyl ether in a separatory funnel. In the four washes that took place, the top organic layer (diethyl ether) was kept. The product was then removed from the diethyl ether via rotary evaporation into a clean pre-weighed 50mL round bottom flask. After beign massed, our highest yield of Compound 2 (172.034g/mol) was 1.7239g. The theoretical yield of Reaction 2 was 2.9780g; therefore the highest percent yield was 57.89%. A ^{1}H NMR was taken to check the structure of this compound, which was found consistent with the assigned structure. The process of Reaction 2 is outlined in Scheme 2.



Scheme 3: Reaction 2 - Neutralization of the salt by removable of proton via addition of a strong base

Reaction 3 – Synthesis of Compound 3

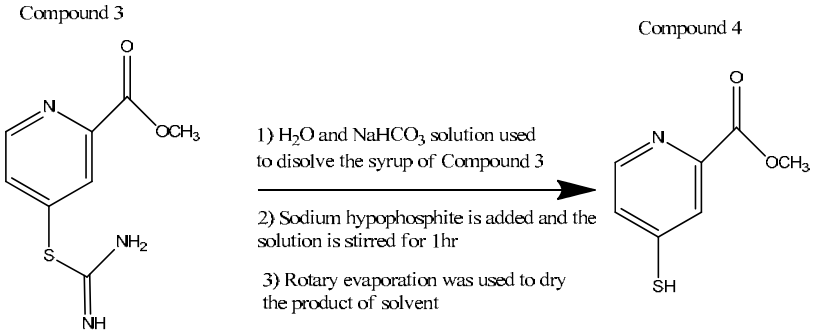
The purpose of Reaction 3 was to substitute the aromatic chlorine with a thiourea group. Reaction 3 began in the same flask containing the dried Compound 2 (1.7239g, 1.002E-2 mol). 14.00mL of methanol was stirred into the flask, dissolving the Compound 2. Once dissolved, thiourea (1.6009g, 76.12, 2.103E-2 mol) was added and stirred into the solution. Next, the solution was brought to reflux (roughly 70^{o}C) for 2 hours. The resulting solution then had the solvent removed by rotary evaporation into a clean pre-massed 50mL round bottom flask. The resulting syrup of Compound 3 (.05855g, 211.240g/mol) was massed and a ^{1}H NMR was taken. The theoretical yield of Reaction3 was 2.1168g; therefore the highest percent yield was 27.66%. The outline of Reaction 3 is seen in Scheme 3.



Scheme 4: Reaction 3 - Subsitution of chlorine with thiourea

Reaction 4 – Synthesis of Compound 4

The purpose of Reaction 4 was to oxidize the thiourea group into a thiol. The syrup of Compound 3 from multiple successful trials of Reaction 3 (.344g, 1.628E-3 mol) was kept in a 50mL round bottom flask. In a separate flask, sodium bicarbonate ($NaHCO_3$, .6010g, 7.153E-3 mol) was dissolved in 9.00mL of water. Once dissolved, the sodium bicarbonate solution was added to the flask containing Compound 3. While stirring, sodium hypophosphite ($NaPO_2H_2$, .2006g, 2.280E-3 mol) was then added to the flask. A slight color change from yellow to orange was noted with this addition. The solution was stirred at room temperature in the hood with a breathing apparatus for one hour. After the hour, solvent was removed via rotary evaporation. The outline of Reaction 4 can be seen in Scheme 4.



Scheme 5: Reaction 4 - Oxidation of the thiourea group into thiol

Thin layer chromatography (TLC), using a 10% methanol and 90% chloroform solution, was then used in order to test the purity of the syrup product. Under ultraviolet light, the TLC test showed three separate bands, or three molecules with varying polarities. In order to purify the three bands, column chromatography was used. A solution of 7% methanol and 93% chloroform allowed for sufficient separation in the bands. Each of the three bands were collected and solvent was removed via rotary evaporation into separate pre-weighted 25mL round bottom flasks. Accordingly, a ^1H NMR was taken of each of the three solid products.

The NMR spectra revealed the three compounds seen in the figure below. Two of the solids were our starting materials (Compound 2 and Compound 3) and the other was Compound 4 (169.20 g/mol, .0101g, 5.969E-5 mol). The three compounds are seen and labeled in Figure 7. The theoretical yield for reaction 3 was .2755g of Reaction 4, meaning our highest percent yield was 3.44%.

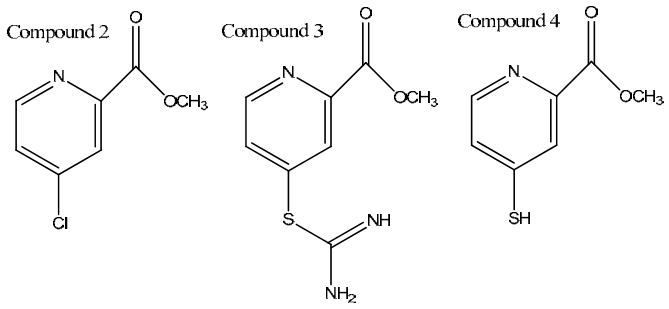


Figure 7: The three molecules purified from column chromatography after Reaction 4

It should be noted that when Reaction 4 was attempted again, Compound 4 was not synthesized. ^{1}H NMR revealed that the band previously corresponding to Compound 4 was now a disulfide. The disulfide was a product of oxidation of two of the thiol groups of Compound being in the presence of oxygen. While being stored in the freezer, Compound 4 had oxidized into its disulfide. Reaction 4 was repeated except under the presence of nitrogen gas, but the disulfide was created again. The ^{1}H NMR of the disulfide is seen in Figure 8.

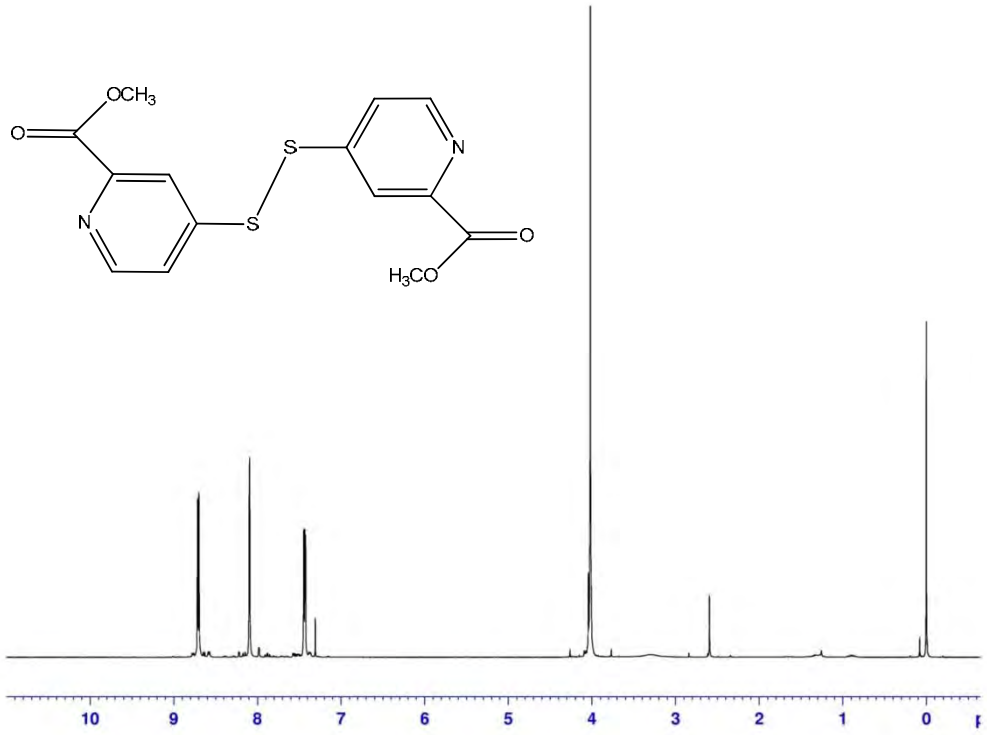
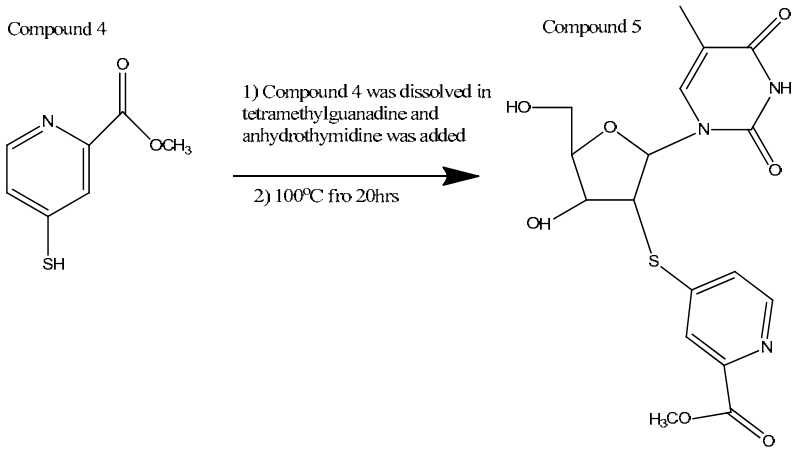


Figure 8: Proton NMR of the disulfide of Compound 4

^{1}H NMR (DMSO, 300MHz): 8.76 (d, 5H, CH); 8.74 (d,5H,CH); 8.21 (s,2H,CH); 7.75 (d,4H, CH); 4.09 (s,8H,CH_3); 2.48 (s, H, DMSO).

Reaction 5 – Synthesis of Compound 5

The purpose of reaction 4 was to attach the nucleoside, thymidine, by synthesizing a thioester. Even though compound 3 could not be isolated, reaction 4 was attempted with the disulfide of compound 3 as the starting material. The 25mL flask containing the disulfide of compound 3 (.0101g, 5.969E-5 mol) was dissolved in tetramethylguanadine (.05055mL, 4.029E-3 mol). Once dissolved, anhydrothymidine (.0956g, 3.980E-4 mol) was stirred into the solution. With the use of an oil bath, condenser, and breathing apparatus, the solution was heated to 100^{o}C for 20 hours. After the 20 hour period, the resulting solution was dried via rotary evaporation. TCL testing in 10% methanol and 90% chloroform showed the presence of one molecule. A ^{1}H NMR was taken for the solid and this spectrum revealed the disulfide as the only present compound. The outline of the intended reaction 4 is seen below in Figure 12. No reaction had taken place and our research was stopped here.



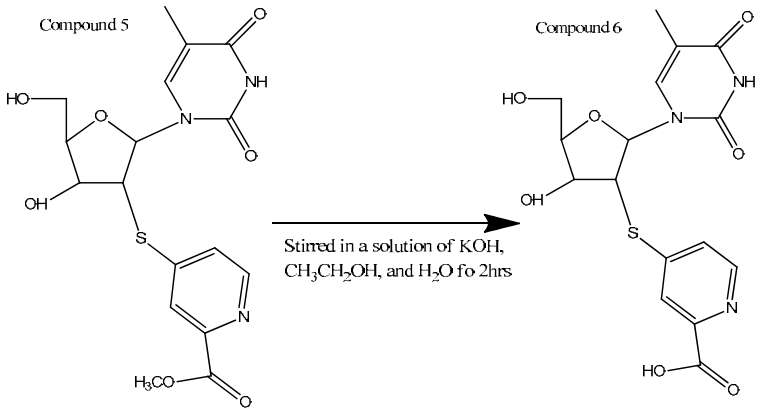
Scheme 6: Reaction 5 – Synthesis of a thioester by attaching thymidine

If we had continued, the following reactions would have been carried out. Refer back to Scheme 1 for the follow reactions.

Reaction 6 – Synthesis of Compound 6

Compound 5 was to be treated with potassium hydroxide, ethanol, and water in the same step. This reaction would have serve as a saponification of the ester attachment made in

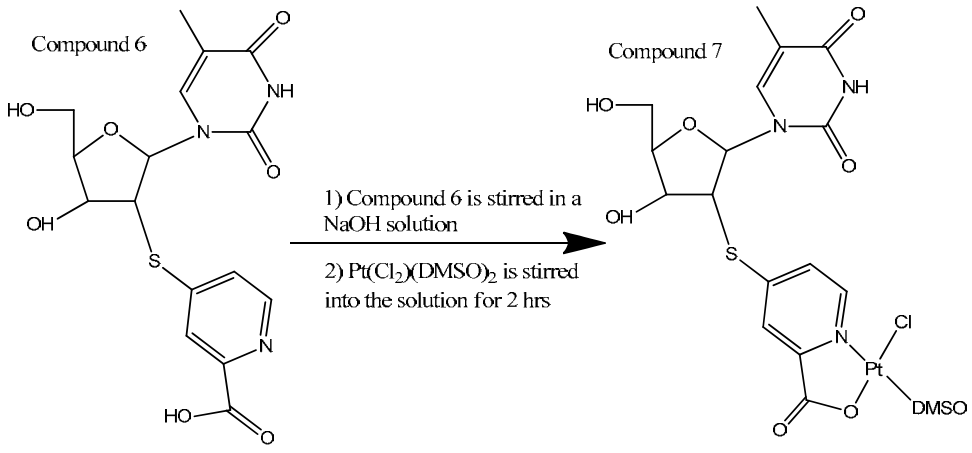
Reaction 2; thus recreating the carboxylic acid attachment. The outline of the proposed Reaction 6 is seen below in Scheme 7.



Scheme 7: Reaction 6 - Recreation of carboxlylic attachment via saponification of the ester attachment

Reaction 7 – Synthesis of Compound 7

Compound 6 will be taken through a two-step reaction designed to bind platinum. First, sodium hydroxide will be used to deprotonate the carboxylic acid attachment of Compound 6. In the second step, cis $PtCl_2(DMSO)_2$, will be added into the solution. One of the two chlorines attached to the platinum would have acted as a leaving group, thus allowing the platinum to bind to both the nitrogen of our attachment ring and the deprotonated oxygen of the carboxylic acid. The outline of the proposed Reaction 6 is seen below in Scheme8.



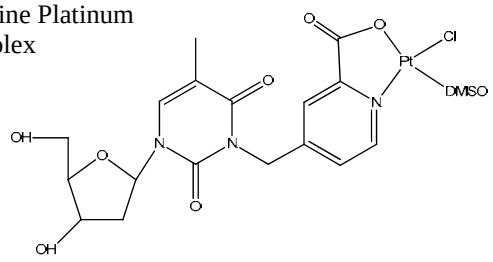
Scheme 8: Reaction 7 - Deprotonating of the carboxylic acid attachment and binding of the platinum ion

Conclusions

The target nucleoside platinum II complex was not successfully synthesized. Even though Reaction 3 was complete, the thiol Compound 3 was oxidized in the presence of oxygen gas; thus creating an unreactive disulfide. Attempts of Reaction 3 under nitrogen gas and Reaction 4 using the disulfide at the reactant both proved ineffective.

Although this target molecule could not be synthesized, other nucleoside platinum complexes could be new target molecules. Specifically, a nucleoside platinum complex that does not involve a thiol intermediate could be sought after. Two examples of new target molecules being explored in Dr. Church's lab include a thymidine and adenosine platinum complex. These structures are shown below in Figure 9.

Thymidine Platinum II Complex



Adenosine Platinum II Complex

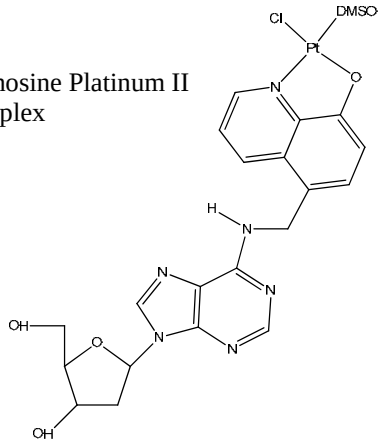


Figure 9: Possible new target nucleoside platinum II complexes for further research

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